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EFFECTS OF ASARONE AND β-ASARONE ON CONDITIONED RESPONSES, FIGHTING BEHAVIOUR AND CONVULSIONS

BY

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In the Ayurvedic system of medicine, the roots and rhizomes of an indigenous Indian plant *Acorus calamus* are used together with the roots of *Rauwolfia serpentina* for treating many mental ailments. The influence of asarone and β-asarone (the *trans* and *cis* forms of 2,4,5-trimethoxy-1-propenyl benzene), two active principles of *Acorus calamus*, when given alone and together with either reserpine or chlorpromazine, has been studied on the conditioned avoidance response of trained rats, on the fighting behaviour of paired mice subjected to mild foot shock and on electro-convulsions. Except for electro-convulsions, asarone in small doses potentiates the effects of reserpine and of chlorpromazine ; β-asarone has no such effect. Estimation of the 5-hydroxytryptamine content of rat brain showed that neither acorus oil nor its active principles increase the concentration of 5-hydroxytryptamine ; nor do these compounds cause an additional decrease in the 5-hydroxytryptamine content of the brains of animals treated with reserpine. It is concluded that the potentiating effect of these principles is unrelated to 5-hydroxytryptamine concentration. In experiments using electro-convulsions, asarone increased the percentage mortality of animals treated with chlorpromazine but not of those treated with reserpine.

The volatile oil obtained by the steam distillation of an indigenous Indian plant *Acorus calamus* possesses interesting actions on the central nervous system (Dandiya & Cullumbine, 1959 ; Dandiya, Cullumbine & Sellers, 1959). The active principles, asarone and β-asarone (*trans* and *cis* forms respectively of 2,4,5-trimethoxy-1-propenyl benzene), have been isolated (Baxter, Dandiya, Kandel, Okany & Walker, 1960). It was reported earlier from this laboratory that these compounds possess many pharmacological properties similar to those of reserpine and chlorpromazine (Dandiya & Sharma, 1962). Each of these compounds prolonged sleep due to hypnotic agents and caused hypothermia. Their influences on spontaneous and learned patterns of behaviour of animals also were closely similar to those due to reserpine. It was also observed that the volatile oil of *Acorus calamus* potentiated both the protective action of reserpine on toxicity due to amphetamine in aggregated mice and also the influence of reserpine in lowering the threshold for seizures induced by leptazol (Dandiya & Cullumbine, 1959). Since the roots and rhizomes of *Acorus calamus* are used in many mental ailments along with the roots of *Rauwolfia serpentina* in the Ayurvedic system of medicine, it was considered worthwhile to investigate the influence of acorus oil and its active principles, asarone and β-asarone, on the action of reserpine and chlorpromazine on behaviour and electro-convulsions.

METHODS

All injections were made intraperitoneally unless otherwise stated.

Conditioned avoidance response. The method employed was similar to that of Cook & Weidley (1957) with minor modifications. Male albino rats weighing between 100 and 150 g were kept in a cage having a grid floor through which an electric current could be passed, and were trained to jump onto the wall of the cage when a bell rang in order to avoid the shock which followed. Two types of responses were studied, namely, the conditioned avoidance response, in which the animal jumped after hearing the bell, and the escape response, in which the animal jumped only after the electric shock; the latter was the unconditioned response. A group of ten or more trained animals which had developed the conditioned avoidance response were used for each experiment. Reserpine 1 mg/kg, chlorpromazine 3 mg/kg, asarone 3 mg/kg and β-asarone 25 mg/kg were administered intraperitoneally. The animals were re-examined 4 hr after the administration of reserpine and 30 min after the other drugs for the exhibition of the above two responses. Observations were made every hour until any effect was over.

The experiment was repeated with reserpine or chlorpromazine given together with either asarone or β-asarone.

Fighting behaviour of mice. Albino mice weighing between 20 and 30 g were used. The technique was similar to that of Tedeschi, Tedeschi, Mucha, Cook, Hattis & Fellows (1959), with minor modifications. The apparatus has parallel copper rods, 4.77 mm in diameter, placed 6.35 mm apart. Pairs of mice were placed on it, covered with a bell jar and given weak electrical shocks using direct current pulses of 0.5 msec duration at 20 shocks/sec from a 60 V supply. When tested in this way at least half of the randomly selected pairs of mice exhibited two to three fighting responses in 1 min. Pairs of fighting mice were selected and kept in individual cages. Different groups of fighting pairs were treated with chlorpromazine (3 mg/kg and 10 mg/kg), reserpine (1 mg/kg and 3 mg/kg), acorus oil (10 mg/kg and 25 mg/kg), asarone (1 mg/kg and 3 mg/kg) and β-asarone (25 mg/kg). The animals were re-examined 4 hr after the administration of reserpine and 30 min after the other drugs for the exhibition of the fighting responses. Pairs of mice not fighting even once in 1 min were taken as showing no response. Other sets of mice received either reserpine or chlorpromazine together with acorus oil, asarone or β-asarone.

In both the above experiments the selection of the doses for combinations of drugs was guided by the results obtained with individual drugs; the doses used were those which just failed to be effective when given alone. Acorus oil, asarone and β-asarone were administered 4 hr after reserpine and 15 min after chlorpromazine.

Estimation of 5-hydroxytryptamine in whole brain of rats. The whole brain was removed 20 hr after the administration of reserpine (1 mg/kg) and 30 min after the administration of acorus oil (100 mg/kg), asarone (25 mg/kg) and β-asarone (25 mg/kg). It was cut into pieces in acetone and allowed to remain in four volumes of acetone for 24 hr for extraction. The acetone layer was decanted and the residue re-extracted with another four volumes of acetone. The acetone extracts were mixed, evaporated to dryness at 35° C and the residue taken up in 0.9% saline. When reserpine was given as well as asarone or β-asarone, these last compounds were administered 20 hr after the reserpine and the brain was removed and extracted 30 min later.

The 5-hydroxytryptamine content of the saline extract was estimated biologically using the rat fundus method of Vane (1957). The fundus strip was suspended in oxygenated Tyrode solution containing hyoscine (10^{-7}) at 37° C. The 5-hydroxytryptamine content of brain extracts of untreated rats was also estimated with each set of experiments to serve as a control.

Electro-convulsions. Convulsions were induced in albino rats weighing between 125 and 150 g by application of a current of 150 mA for 0.2 sec through ocular electrodes. The prevention of extensor tonic spasm and death was accepted as the criterion of protection from electro-shock seizure. The prolongation of extensor tonic spasm or the increase in percentage

mortality of animals in 24 hr was taken as the criterion in estimating the potentiation of electro-convulsions by the compounds. The compounds tested were reserpine (3 mg/kg), chlorpromazine hydrochloride (10 mg/kg), asarone (25 mg/kg) and β-asarone (25 mg/kg), alone or together. A dose of 25 mg of asarone or of β-asarone per kg body weight was used with reserpine (3 mg/kg) or chlorpromazine (10 mg/kg).

RESULTS

Administration of reserpine (1 mg/kg) and of chlorpromazine (3 mg/kg) caused mild sedation. The degree of sedation after asarone (3 mg/kg) was similar to that after chlorpromazine (3 mg/kg); β-asarone (25 mg/kg) caused no sedation. When asarone (3 mg/kg) was given with either reserpine (1 mg/kg) or chlorpromazine (3 mg/kg) the sedation was much greater than when these two last drugs were given alone, but the righting reflex was not lost. Asarone (25 mg/kg) produced considerable sedation but failed to cause sleep or to abolish the righting reflex; but when this dose of asarone was given to animals previously treated with reserpine (1 mg/kg) or chlorpromazine (3 mg/kg) they lost the righting reflex. Acorus oil (50 mg/kg) had a similar though less pronounced effect when given after reserpine. β-Asarone, even in a dose of 25 mg/kg, failed to modify sedation due to reserpine or chlorpromazine.

Conditioned avoidance response

Chlorpromazine (3 mg/kg) and reserpine (1 mg/kg) were effective in blocking the conditioned avoidance response in approximately 45% of the animals. However, when asarone (3 mg/kg), which given alone abolished the response in 30% of the animals, was administered to animals treated with chlorpromazine or reserpine, both this response and the escape response were completely abolished (with chlorpromazine) or nearly completely (with reserpine) without motor inactivation (Table 1). Asarone also prolonged the action of both chlorpromazine and of reserpine on

TABLE 1

EFFECTS OF ASARONE AND β-ASARONE ALONE AND AFTER CHLORPROMAZINE AND RESERPINE ON THE CONDITIONED AVOIDANCE RESPONSE (CAR) AND THE ESCAPE RESPONSE OF TRAINED RATS

The results of treatment with asarone after administration of chlorpromazine or reserpine were compared with those obtained with the respective drug alone in applying the χ^2 test of probability

Drug	Dose (mg/kg)	No. of rats	No. of rats showing loss of CAR	No. of rats showing loss of escape response	Duration of action (hr)
Chlorpromazine	3	30	13	0	5–6
Reserpine	1	30	14	2	6–8
Asarone	3	10	3	0	1–2
β-Asarone	25	10	3	0	4–6
Chlorpromazine + asarone	3 +3	10	10 ($P<0.025$)	10 ($P<0.001$)	8–10
Chlorpromazine + β-asarone	3 +25	10	4	0	1
Reserpine + asarone	1 +3	10	9 ($P<0.02$)	9 ($P<0.001$)	10–12
Reserpine + β-asarone	1 +25	10	6	2	4–6

the conditioned avoidance response. β-Asarone, given alone, also abolished the response in 30% of animals but, unlike asarone, had little effect on animals treated with chlorpromazine or reserpine.

Fighting behaviour of mice

The results are given in Table 2. Chlorpromazine (3 mg/kg) and reserpine (1 mg/kg) failed to influence the fighting behaviour of mice, but in larger doses chlorpromazine (10 mg/kg) and reserpine (3 mg/kg) suppressed this response in 70% and 46% of the animals respectively. Acorus oil (10 and 25 mg/kg) suppressed the fighting response in 8 and 50% of the animals respectively, and asarone (1 and 3 mg/kg) suppressed it in 20 and 80% respectively. When asarone (1 mg/kg) was given after reserpine (1 mg/kg) the response was not suppressed, but when this dose of asarone was given after reserpine (3 mg/kg) or chlorpromazine (3 mg/kg), there was 100% ($P<0.001$) and 90% ($P<0.006$) suppression respectively. Some ataxia was produced by reserpine and by chlorpromazine, but it was not intensified by asarone. β-Asarone (20 mg/kg) had no effect whether given alone or after reserpine or chlorpromazine.

TABLE 2

EFFECTS OF ACORUS OIL, ASARONE, AND β-ASARONE ALONE AND AFTER ADMINISTRATION OF CHLORPROMAZINE AND RESERPINE ON THE FIGHTING BEHAVIOUR OF PAIRS OF MICE SUBJECTED TO MILD FOOT-SHOCK

The results of treatment with asarone after administration of chlorpromazine or reserpine were compared with those obtained with the respective drug alone in applying the χ^2 test of probability

Drug	Dose (mg/kg)	No. of fighting pairs	No. of pairs not fighting after treatment
Chlorpromazine	3	10	0
	10	10	7
Reserpine	1	6	0
	3	13	6
Acorus oil	10	12	1
	25	12	6
Asarone	1	10	2
	3	10	8
β-Asarone	20	10	0
Chlorpromazine +asarone	3 +1	10	9 ($P<0.001$)
Chlorpromazine +β-asarone	3 +20	10	1
Reserpine +acorus oil	3 +10	12	10
Reserpine +asarone	1 +1	9	0
Reserpine +asarone	3 +1	10	10 ($P<0.006$)
Reserpine +β-asarone	1 +20	9	0

5-Hydroxytryptamine content of rat brain

Reserpine (1 mg/kg) reduced the 5-hydroxytryptamine content of rat brain to about 30% of the control value but neither acorus oil nor its active principles

TABLE 3

EFFECTS OF ACORUS OIL, ASARONE, AND β-ASARONE ALONE AND AFTER ADMINISTRATION OF RESERPINE ON THE 5-HYDROXYTRYPTAMINE (5-HT) CONTENT OF RAT BRAIN

Values are means±standard errors

Drug	Dose (mg/kg)	No. of rats	5-HT content of brain (μg/g)
None		12	0·421±0·061
Reserpine	1	8	0·135±0·018
Acorus oil	100	6	0·428±0·051
Asarone	25	6	0·448±0·057
β-Asarone	25	6	0·392±0·060
Reserpine + asarone	1 +25	4	0·182±0·021
Reserpine + β-asarone	1 +25	5	0·168±0·018

altered the content significantly (Table 3). After reserpine had been given, asarone or β-asarone caused no further decrease in 5-hydroxytryptamine level; rather the level was slightly higher than when reserpine was given alone.

Electro-convulsions

All control animals showed extensor tonic spasm but none died (Table 4). Asarone (25 mg/kg) protected 44% of the animals as indicated by the absence of extensor tonic spasm. β-Asarone, in the same dose, increased the duration of extensor tonic spasm. Chlorpromazine (10 mg/kg) killed 10% of the animals and, when this dose of chlorpromazine was followed by asarone (25 mg/kg), the death rate rose to 60%. β-Asarone (25 mg/kg) caused no deaths of animals treated with chlorpromazine. The mortality due to reserpine (3 mg/kg) alone was 30%, and this was unchanged when asarone (25 mg/kg) was given afterwards.

TABLE 4

EFFECTS OF ASARONE AND β-ASARONE ALONE AND AFTER ADMINISTRATION OF RESERPINE OR CHLORPROMAZINE ON ELECTRO-SHOCK SEIZURES IN RATS

The results of treatment with asarone and chlorpromazine were compared with that following chlorpromazine alone and the probability was calculated using the χ^2 test

Drug	Dose (mg/kg)	No. of rats	No. of rats showing extensor tonic spasm	Average duration of extensor tonic spasm (sec)	No. of deaths
None		10	10	5·6	0
Reserpine	3	10	10	3·2	3
Chlorpromazine	10	10	10	9·9	1
Asarone	25	9	5	3·5	0
β-Asarone	25	10	10	11·0	0
Reserpine + asarone	3 +25	10	10	4·6	3
Reserpine + β-asarone	3 +25	10	10	4·0	0
Chlorpromazine + asarone	10 +25	10	10	7·0	6 ($P<0·01$)
Chlorpromazine + β-asarone	10 +25	10	10	8·5	0

DISCUSSION

Reserpine and chlorpromazine have been reported by Cook & Weidley (1957) and by Courvoisier, Fournel, Ducrot, Kolsky & Koetschet (1953) as showing a specific blockade of the conditioned avoidance response without affecting the unconditioned response. Acorus oil and its active principles, asarone and β-asarone, also have similar actions. Acorus oil and asarone in sub-threshold doses potentiate the actions of chlorpromazine and reserpine on the conditioned avoidance response of trained rats. Asarone, with either reserpine or chlorpromazine, not only potentiates the effect on this response but also makes the blockade non-specific, that is, the unconditioned response is also abolished. Barbiturates abolish these responses in doses which produce motor inactivation (Cook & Weidley, 1957); but, with the present drug combinations, the effect cannot be attributed to motor inactivation because, though there was increased sedation in animals treated with the combination of drugs, motor activity was not abolished.

The fighting behaviour of paired mice was found to be a more sensitive index for evaluation of the tranquillizing property of acorus oil and asarone than the conditioned avoidance response. It has been reported by Tedeschi *et al.* (1959) that both reserpine and chlorpromazine abolished this response only in doses which caused motor inactivation. In our experiments, acorus oil and asarone, when given in sub-threshold doses after a sub-threshold dose of reserpine or chlorpromazine, suppressed fighting behaviour without much influencing sedation or motor activity.

Malhotra, Prasad, Dhalla & Das (1961) reported that 5-hydroxytryptamine is depleted from the brains of rats by acorus oil. In our experiments no such effect was observed either with the oil or its active principles. Asarone and β-asarone also failed to increase depletion in animals treated with reserpine. It can, therefore, be concluded that the central nervous action of acorus oil and its two active principles, whether they are given alone or after reserpine, is unrelated to their effect on the level of 5-hydroxytryptamine in the brain. This view is supported by the observation that the action of chlorpromazine, which does not itself affect the level of 5-hydroxytryptamine in the brain, was also potentiated by asarone in two experiments.

In electro-shock seizures asarone increased the percentage mortality of rats previously treated with chlorpromazine, but did not influence the mortality of rats treated with reserpine, while β-asarone did not greatly influence the action of either drug in this experiment, Since asarone (25 mg/kg), alone, protected against electroconvulsions, and β-asarone in the same dose facilitated them, one might expect some reduction in mortality by asarone and some increase in mortality by β-asarone in animals treated with chlorpromazine. The failure of asarone and of β-asarone to influence the action of chlorpromazine and of reserpine in the expected manner cannot be explained at present.

It has already been reported (Dandiya & Sharma, 1962) that β-asarone shares with asarone many common pharmacological actions. Each compound prolongs sleep due to barbiturates or alcohol, causes hypothermia, has a taming influence on hostile cats and shows a specific blockade of the conditioned avoidance response in trained rats. However, in other experiments, namely those in which convulsions

were induced either by electro-shocks or by leptazol, while asarone had a protective action, β-asarone facilitated convulsions (Dandiya & Sharma, 1962). In the present study, while the effects of reserpine and chlorpromazine on the conditioned avoidance response in trained rats and the fighting response of paired mice subjected to mild foot shock were potentiated by asarone, β-asarone failed to influence these actions. Acorus oil had practically all the actions of asarone ; this could be due to the presence of more asarone than β-asarone in the oil. The differences in some of the actions of these two compounds, which possess the same chemical structure and differ only in configuration, may be due to the failure of β-asarone to act on all receptor sites which are successfully occupied by asarone.

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